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Role of regulatory T cells in tolerance to coagulation factors

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Summary

The immune response to coagulation factors VIII or IX, in particular formation of inhibitory antibodies, complicates treatment of hemophilia. Therefore, a number of recent studies in animal models have explored novel approaches toward induction of immune tolerance in protein or gene replacement therapy. Strong evidence has emerged that regulatory T cells (Treg) are an important component of the mechanism by which tolerance is maintained and inhibitor formation, a T help dependent response, is prevented. Limited data in patients also support this concept. In particular, $CD4^+CD25^+FoxP3^+$ Treg, whether naturally occurring or induced, have been invoked in suppression of antibody and of cytotoxic T lymphocyte responses to the therapeutic clotting factor. This review summarizes the data on this emerging concept of Treg-mediated regulation of the immune response in treatment of hemophilia, strategies and mechanisms of Treg induction and function, and the implications for development of immune tolerance protocols.

Keywords

tolerance; factor IX; factor VIII; hemophilia; regulatory T cells

Introduction

Hemophilia is an X-linked bleeding disorder caused by mutations in coagulation factor VIII (hemophilia A) or IX (hemophilia B). Severe disease is defined by protein levels below 1% of normal, and typically results in frequent spontaneous joints and soft tissue bleeding. Bleeding into critical closed spaces (e.g. intracranial) can be fatal. Conventional therapy for hemophilia patient is intravenous infusion of plasma-derived or recombinant F.VIII or F.IX protein. In recent years, successes in the treatment of animals with genetic disease have been reported using gene therapies, which are now being explored in clinical trials [1]. In general, an immune response to the therapeutic protein represents a serious complication of treatment. In hemophilia, formation of antibodies that are inhibitory to factor activity is a major concern. In protein therapy, the overall incidence of inhibitor formation is ~3-4% in hemophilia B and 20-30% in hemophilia A (10-20% and 30-40% in severe disease, respectively). The risk in gene therapy remains to be defined, but is in part influenced by the gene transfer vector and target organ. Formation of inhibitors is dependent on T help, and subjects with gene deletion or nonsense mutations are more likely to form inhibitors compared to missense mutations. Protocols exist to treat inhibitors using frequent intravenous infusion of high dose factor, often in combination with immunoglobulin infusion and immune suppression.

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It has become obvious that immune regulation is an important and integral component of tolerance to self-antigens and of many forms of induced tolerance. Recent studies provide a rapidly growing body of evidence that regulatory T cells (Treg) play a crucial role in tolerance to coagulation factors delivered by means of gene transfer. Evidence for involvement of Treg in controlling the pathogenesis of inhibitor formation in patients has also been provided. This review seeks to provide an overview of these data and their implications for treatment of hemophilia.

Treg -mediated suppression

Mechanisms of Treg function involve many diverse pathways, molecules, and processes. In addition, a number of different subsets of T cells with suppressor activities have been described, including $CD4^+CD25^+FoxP3^+$ Treg, IL-10-producing Tr1 cells, transforming growth factor-β producing Th3 cells, $CD8^+$ Treg, NK-T cells, $CD4^-CD8^-$T cells, and γδ T cells [2,3]. $CD4^+CD25^+FoxP3^+$ Treg originate during thymic T cell development (also referred to as 'natural' Treg) and constitutively express CD25, the α-chain of the IL-2 receptor, GITR, CTLA-4, and transcription factor FoxP3, the master switch for their development. However, these Treg may also be induced from conventional T cells in the periphery [2]. While activation of Treg requires antigen-specific signaling through their T cell receptor, it is thought that suppression occurs in a nonspecific fashion. Therefore, Treg with one antigen specificity can suppress effector T cells (Teff) with distinct antigen specificities (bystander suppression). Furthermore, existing Treg can promote the outgrowth of a new Treg, which can be converted from $CD4^+CD25^-$ cells and may have antigen specificities distinct from the original Treg population.

Treg may suppress Teff through a variety of mechanisms [2]. For example, Treg can suppress the proliferation and cytokine production of Teff and can prevent $CD8^+$ cells from differentiating into fully functional cytolytic cells, which is reversible in the absence of Treg. While Treg activity may be exerted by cell contact-dependent mechanisms involving antigen presenting cells (APCs) or by secretion of cytokines, it is also suggested that Treg suppress through direct contact with Teff. Other studies have shown that Treg can kill Teff or APCs directly through the release of granzyme B and perforin, or alter T cell differentiation. For example, $CD4^+$ Teff may differentiate into IL-10- or TGF-β-producing adaptive Treg in the presence of Treg. Another important aspect is the ability of Treg to modulate APC function, including down-regulation of antigen-presentation and promotion of secretion of suppressive factors by dendritic cells (DCs). Treg can stimulate DCs to up regulate indoleamine 2,3-dioxygenase, a potent immunosuppressive enzyme, as well as activate TGF-β on the surfaces of DCs, which may facilitate the conversion of Teff into Treg. Thus, Treg promote tolerogenic rather than immunogenic antigen presentation, thereby creating an immune suppressive microenvironment and expansion of the Treg repertoire. A variety of immune suppressive molecules derived from Treg have been identified, including cytokines IL-10, TGF-β, and IL-35. Heme oxigenase-1, which catalyzes the formation of carbon monoxide through heme degradation, and generation of adenosine by CD39 and CD73 have also been linked to Treg function [2].

Hepatic gene transfer induces Treg and immune tolerance to factor IX

Viral gene transfer, as currently tested in clinical trials for hemophilia, can direct high levels of transgene expression [4]. While inhibitor formation has been found in some pre-clinical gene transfer studies, sustained expression of F.IX has been reported in a number of animal studies based on liver-directed adeno-associated viral (AAV) gene transfer, which often induces immune tolerance to F.IX [1]. F.IX-specific tolerance requires a minimal level of expression (30-50 ng /ml plasma), which is determined the vector dose, promoter, and

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mouse strain [5]. Mice transgenic for a T cell receptor (DO11.10) to ovalabumin (ova) provided direct evidence for induction of transgene product-specific $CD4^+$ T-cell tolerance by hepatic AAV gene transfer [6]. Interestingly, in addition to deletion of $CD4^+$ T cells and T cell anergy, the percentage of ova-specific Treg increased after gene transfer. Responsiveness to ova was partially restored after removing $CD4^+CD25^+$ T cells. DO11.10-tg RAG-$2^{-/-}$ mice, which lack Treg, were useful in demonstrating that hepatic AAV gene transfer induces transgene product-specific $CD4^+CD25^+$ Treg, which are similar to natural Treg for their expression of FoxP3, GITR, and CTLA-4 [7]. The first 2 months following gene transfer represent the induction phase of immune tolerance, during which the Treg frequency increases.

Induction of Treg by F.IX gene transfer can be demonstrated by adoptive transfer of splenic $CD4^+$ T cells from mice tolerized to F.IX to naïve animals of the same strain. In these recipient mice, antibody formation and inflammatory T cell responses to F.IX-transduced liver were suppressed [5,6,8]. $CD4^+$ T cells expressing the surface markers CD25 and GITR mediated this suppression [7]. Depletion of $CD4^+CD25^+$ cells caused antibody formation against F.IX upon hepatic gene transfer. In a study in non-human primates, to test suppression of immune responses to the AAV vector, a 3-drug regimen consisting of mycophenolate mofetil, sirolimus, and the Daclizumab (a monoclonal anti-CD25) was administered. However, this protocol resulted in formation of inhibitors against human F.IX following hepatic administration of the vector. In contrast, a 2-drug regimen (excluding anti-CD25) did not [9]. Taken together, these data indicate a requirement and a crucial role for $CD4^+CD25^+FoxP3^+$ Treg in tolerance to F.IX.

Treg in B-cell mediated F.VIII gene transfer

$CD4^+CD25^+$ Treg are also required for B cell-mediated tolerance induction to F.VIII. Scott and colleagues showed that tolerance induction to F.VIII by a retroviral gene transfer of immunodominant F.VIII-derived A2 and C2 domains presented by B cells as Ig fusion proteins depended on Treg [10]. In a subsequent study to track Treg in B-cell transduced animals, reporter mice that express GFP in $FoxP3^+$ cells were created and crossed with DO11.10-tg or DO11.10-tg Rag $2^{-/-}$ mice [11]. When FoxP3-GFP/DO11.10-tg mice are treated with fusion ova-Ig transduced B cells, a significant increase in antigen-specific Treg was found. Furthermore, when the same treatment was applied to FoxP3-GFP/DO11.10-transgenic $Rag^{-/-}$ mice, which lack natural Treg, $FoxP3^+$ cells increased to 4% of the total T cell population.

Treg in plasmid-mediated F.VIII gene transfer

Miao *et al*. have shown that hepatic non-viral (plasmid-mediated) F.VIII gene transfer to hemophilia A mice causes a potent inhibitor response. However, plasmid administration to transgenic hemophilia A mice, over-expressing FoxP3, did not induce antibodies against F.VIII [12]. Suppression of inhibitor formation was adoptively transferable to non-transgenic hemophilia A mice. Alternatively, short-term therapy with an anti-ICOS monoclonal antibody to transiently block the inducible costimulator/inducible costimulator ligand (ICOS/ICOSL) signaling pathway led to tolerance in plasmid-treated hemophilia A mice. Treatment resulted in depletion of $ICOS^+CD4^+$ T cells and activation of $CD4^+CD25^+Foxp3^+$ Treg capable of suppressing immune responses to F.VIII [13].

Treg in the treatment of hemophilia with protein replacement therapy

Immune tolerance induction therapy (ITI) using frequent administration of F.VIII over a period of several weeks to 2 or more years is the only strategy that has been proven to successfully combine the eradication of inhibitors and the induction of tolerance to F.VIII in

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patients. However, little is known about the mechanisms responsible for elimination of inhibitors and induction of long-lasting immune tolerance. Similarly, information on phenotypic and functional changes of F.VIII-specific lymphocytes involved in immune responses and regulation during ITI is limited. Nonetheless, based on findings in animal models, it has been speculated that chronic exposure of the immune system to F.VIII under non-inflamed conditions leads to the induction of Treg [14]. Subsequent lack of T help would prevent the differentiation of B cells into antibody-producing plasma cells, and eventually lead to the deletion of these cells or to B cell anergy. Hausl *et al*. showed that the re-stimulation and differentiation of FVIII-specific memory B cells in hemophilia A mice is sensitive to increasing doses of FVIII, with a threshold dose for optimal stimulation of memory responses [15]. F.VIII doses above this threshold irreversibly inhibit rather than stimulate memory B cells. Selective inhibition of memory B cells could be an important early event in the induction of tolerance to F.VIII in patients with inhibitors [14]. This could lead to a deficiency of APCs critical for the re-stimulation of F.VIII-specific Teff, leading to Treg induction [16,17]. Some ITI protocols include infusion of immunoglobulin. Recently, Ephrem *et al*. demonstrated that intravenous immunoglobulin expands Treg and enhances their function [18].

Furthermore, highly promiscuous MHC class II epitopes in the Fc fragment of IgG that are capable of specifically activating $CD4^{+}CD25^{hi}FoxP3^{+}$ Treg [19]. These findings shed new light on the previously elusive mechanism of action of IVIg.

Treg in novel pre-clinical immune tolerance protocols

Induction of immune tolerance to coagulation factors by mucosal exposure is in part mediated by Treg and involves up-regulation of IL-10 and TGF-β [20,21]. Hemophilia A mice pre-treated with oral or nasal administration of C2 domain have reduced inhibitor titers against F.VIII. Similarly, inhibitor formation against F.IX in hemophilia B mice can be prevented by repeated nasal administration of a dominant $CD4^{+}$ T cell epitope. Recently, Waters *et al*. tested the efficacy of Non-Fc-receptor binding anti-CD3 to prevent F.VIII inhibitor formation in hemophilia A mice [22]. A short course of low-dose anti-CD3 increased the proportion of Treg and potently prevented the production of antibodies against F.VIII. Depleting $CD4^{+}CD25^{+}$ cells during anti-CD3 therapy completely ablated tolerance to F.VIII. Tolerized mice contained higher numbers of $CD4^{+}GITR^{+}$ and $CD4^{+}FoxP3^{+}$ cells. In addition, an increase in $CD4^{+}CD25^{+}CTLA\text{-}4^{+}$ and $CD4^{+}CD25^{+}mTGF\text{-}\beta1^{+}$ cells was observed. *In vitro* cytokine profiling demonstrated polarization toward a Th1-immune response. These findings indicate that anti-CD3 induces tolerance to FVIII, and that the mechanisms regulating this response involve several distinct regulatory T-cell lineages and changes in cytokine production.

Treg in the pathogenesis of inhibitor formation

Recently, James *et al*. characterized T cell responses of a patient with mild hemophilia A (missense genotype A2201P) for one year following his initial inhibitor response, with the goals to define primary epitopes and their MHC Class II restriction [23]. This patient developed a high-titer inhibitor (250 BU/ml) that declined over time to 8 BU/ml. His clotting activity was initially impaired (3%), but returned to baseline (8-10%) within 4 weeks. Interestingly, the responsiveness of CD25-depleted $CD4^{+}$ cells to an A2201 peptide was enhanced 11 and 19 weeks following inhibitor detection, suggesting involvement of Treg in modulating the immune response [23]. Treg have also been shown to suppress F.VIII-induced proliferation in healthy individuals [24].Therefore, depletion of Treg may enhance F.VIII-specific T-cell responses in non-hemophilic individuals.

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In summary, Treg are a critical component of self-tolerance and many forms of induced tolerance to exogenous antigens, including tolerance to coagulation factors in gene- or protein-based therapies. Moreover, Treg suppress B and T cell responses to coagulation factors and cells expressing these therapeutic proteins. Specific routes of antigen administration or expression and a number of immune modulatory protocols are at least in part successful in achieving tolerance because of induction or recruitment of Treg. Development of novel therapies and immune tolerance protocols can therefore be designed to take advantage of immune regulation.

References

1. Herzog RW, Cao O, Hagstrom JN, Wang L. Gene therapy for treatment of inherited haematological disorders. Expert Opin Biol Ther. 2006; 6:509–22. [PubMed: 16610980]
2. Tang Q, Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. Nat Immunol. 2008; 9:239–44. [PubMed: 18285775]
3. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell. 2008; 133:775–87. [PubMed: 18510923]
4. Cao O, Herzog RW, Hagstrom JN, Wang L. Gene therapy for treatment of inherited haematological disorders. Expert Opin Biol Ther. 2006; 6:509–22. [PubMed: 16610980]
5. Mingozzi F, Liu YL, Dobrzynski E, Kaufhold A, Liu JH, Wang Y, Arruda VR, High KA, Herzog RW. Induction of immune tolerance to coagulation factor IX antigen by in vivo hepatic gene transfer. J Clin Invest. 2003; 111:1347–56. [PubMed: 12727926]
6. Dobrzynski E, Mingozzi F, Liu YL, Bendo E, Cao O, Wang L, Herzog RW. Induction of antigen-specific CD4+ T-cell anergy and deletion by in vivo viral gene transfer. Blood. 2004; 104:969–77. [PubMed: 15105293]
7. Cao O, Dobrzynski E, Wang L, Nayak S, Mingle B, Terhorst C, Herzog RW. Induction and role of regulatory CD4+CD25+ T cells in tolerance to the transgene product following hepatic in vivo gene transfer. Blood. 2007; 110:1132–40. [PubMed: 17438084]
8. Dobrzynski E, Fitzgerald JC, Cao O, Mingozzi F, Wang L, Herzog RW. Prevention of cytotoxic T lymphocyte responses to factor IX-expressing hepatocytes by gene transfer-induced regulatory T cells. Proc Natl Acad Sci U S A. 2006; 103:4592–7. [PubMed: 16537361]
9. Mingozzi F, Hasbrouck NC, Basner-Tschakarjan E, Edmonson SA, Hui DJ, Sabatino DE, Zhou S, Wright JF, Jiang H, Pierce GF, Arruda VR, High KA. Modulation of tolerance to the transgene product in a nonhuman primate model of AAV-mediated gene transfer to liver. Blood. 2007; 110:2334–41. [PubMed: 17609423]
10. Lei TC, Scott DW. Induction of tolerance to factor VIII inhibitors by gene therapy with immunodominant A2 and C2 domains presented by B cells as Ig fusion proteins. Blood. 2005; 105:4865–70. [PubMed: 15769892]
11. Skupsky, Jonathan EF.; Scott, David W. Role of adaptive regulatory T cells in the induction of tolerance to FVIII. Blood. 2008; 112:377.
12. Harmeling BRZS, Torgerson T, Chen L, Peng B, Ochs HD, Rawlings DJ, Miao CH. Regulation of immune responses against factor VIII following nonviral gene transfer in hemophilia A and FoxP3 transgenic mouse models. Mol Ther. 2006; 13:S33.
13. Peng B, Ye P, Blazar BR, Freeman GJ, Rawlings DJ, Ochs HD, Miao CH. Transient blockade of the inducible costimulator pathway generates long-term tolerance to factor VIII after nonviral gene transfer into hemophilia A mice. Blood. 2008; 112:1662–72. [PubMed: 18574023]
14. Reipert BM, van Helden PM, Schwarz HP, Hausl C. Mechanisms of action of immune tolerance induction against factor VIII in patients with congenital haemophilia A and factor VIII inhibitors. Br J Haematol. 2007; 136:12–25. [PubMed: 17222196]
15. Hausl C, Ahmad RU, Sasgary M, Doering CB, Lollar P, Richter G, Schwarz HP, Turecek PL, Reipert BM. High-dose factor VIII inhibits factor VIII-specific memory B cells in hemophilia A with factor VIII inhibitors. Blood. 2005; 106:3415–22. [PubMed: 16091456]
16. Apostolou I, von Boehmer H. In vivo instruction of suppressor commitment in naive T cells. J Exp Med. 2004; 199:1401–8. [PubMed: 15148338]

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17. Chen TC, Cobbold SP, Fairchild PJ, Waldmann H. Generation of anergic and regulatory T cells following prolonged exposure to a harmless antigen. J Immunol. 2004; 172:5900–7. [PubMed: 15128770]
18. Ephrem A, Chamat S, Miquel C, Fisson S, Mouthon L, Caligiuri G, Delignat S, Elluru S, Bayry J, Lacroix-Desmazes S, Cohen JL, Salomon BL, Kazatchkine MD, Kaveri SV, Misra N. Expansion of CD4+CD25+ regulatory T cells by intravenous immunoglobulin: a critical factor in controlling experimental autoimmune encephalomyelitis. Blood. 2008; 111:715–22. [PubMed: 17932250]
19. De Groot AS, Moise L, McMurry JA, Wambre E, Van Overtvelt L, Moingeon P, Scott DW, Martin W. Activation of natural regulatory T cells by IgG Fc-derived peptide “Tregitopes”. Blood. 2008; 112:3303–11. [PubMed: 18660382]
20. Cao O, Armstrong E, Schlachterman A, Wang L, Okita DK, Conti-Fine B, High KA, Herzog RW. Immune deviation by mucosal antigen administration suppresses gene-transfer-induced inhibitor formation to factor IX. Blood. 2006; 108:480–6. [PubMed: 16543469]
21. Rawle FE, Pratt KP, Labelle A, Weiner HL, Hough C, Lillicrap D. Induction of partial immune tolerance to factor VIII through prior mucosal exposure to the factor VIII C2 domain. J Thromb Haemost. 2006; 4:2172–9. [PubMed: 16824190]
22. Walters S, Webster KE, Sutherland A, Gardam S, Groom J, Liuwantara D, Marino E, Thaxton J, Weinberg A, Mackay F, Brink R, Sprent J, Grey ST. Increased CD4+Foxp3+ T cells in BAFF-transgenic mice suppress T cell effector responses. J Immunol. 2009; 182:793–801. [PubMed: 19124722]
23. James EA, Kwok WW, Ettinger RA, Thompson AR, Pratt KP. T-cell responses over time in a mild hemophilia A inhibitor subject: epitope identification and transient immunogenicity of the corresponding self-peptide. J Thromb Haemost. 2007; 5:2399–407. [PubMed: 18034765]
24. Kamate C, Lenting PJ, van den Berg HM, Mutis T. Depletion of CD4+/CD25high regulatory T cells may enhance or uncover factor VIII-specific T-cell responses in healthy individuals. J Thromb Haemost. 2007; 5:611–3. [PubMed: 17155960]